Recent advances in antiviral drug development towards dengue virus

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Recent advances in antiviral drug development towards dengue virus

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Despite the high disease burden of dengue virus, there is no approved antiviral treatment or broadly applicable vaccine to treat or prevent dengue virus infection. In the last decade, many antiviral compounds have been identified but only few have been further evaluated in pre-clinical or clinical trials. This review will give an overview of the direct-acting and host-directed antivirals identified to date. Furthermore, important parameters for further development that is, drug properties including efficacy, specificity and stability, pre-clinical animal testing, and combinational drug therapy will be discussed.

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Introduction

Dengue virus (DENV) is the most important mosquito-borne viral pathogen worldwide. Each year, an estimated 400 million people are infected leading to approximately 25 000 deaths [1•]. Within the last decades, the virus has drastically re-emerged causing large outbreaks in Africa, South-east Asia, the Americas and even some parts of Europe [2,3]. To date, the virus is endemic in more than 100 countries worldwide [1•]. In endemic countries, most DENV cases are reported in infants and young children. Given the high disease burden and the lack of a broadly applicable vaccine, there is an urgent need for an effective antiviral compound to treat DENV infection [4,5].

DENV is an enveloped single-stranded positive-sense RNA virus, which belongs to the family of *Flaviviridae*. There are 4 antigenically distinct serotypes (DENV 1-4). The first step in infection involves the interaction of the virus particle with the host cell. The DENV envelope (E) glycoprotein is described to bind various host cell receptors, such as glycosaminoglycans (GAGs), dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) and T-cell immunoglobulin and mucin domain (TIM). Thereafter, the virus is internalized via receptor-mediated endocytosis [6•,7]. The low pH within the endosomal vesicle then triggers conformational changes in the E glycoprotein, leading to membrane fusion and the subsequent release of the nucleocapsid into the cytoplasm. Upon nucleocapsid disassembly, the viral RNA is translated into one polyprotein that is ultimately cleaved into 3 structural proteins (Capsid (C),membrane (M) and E) and 7 non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) [2]. The NS proteins exploit the cellular lipid metabolism and induce a re-organisation of the ER membrane to form replicative complexes consisting of double-membrane vesicles where viral RNA replication occurs. Various cellular enzymes such as α-glucosidases and kinases assist in RNA replication within these vesicles and subsequent protein translation and folding. Newly generated genomic RNA is packaged by multiple copies of the C protein and the nucleocapsid then buds into the endoplasmic reticulum (ER) lumen to form an enveloped immature virion. From there, virions are transported through the secretory pathway where the E and M proteins undergo post-translational modifications and conformational changes, including the cleavage of precursor M to its mature form by the host cell protease furin. Progeny virus release occurs via exocytosis [2]. A schematic overview of the replication cycle is presented in Figure 1.

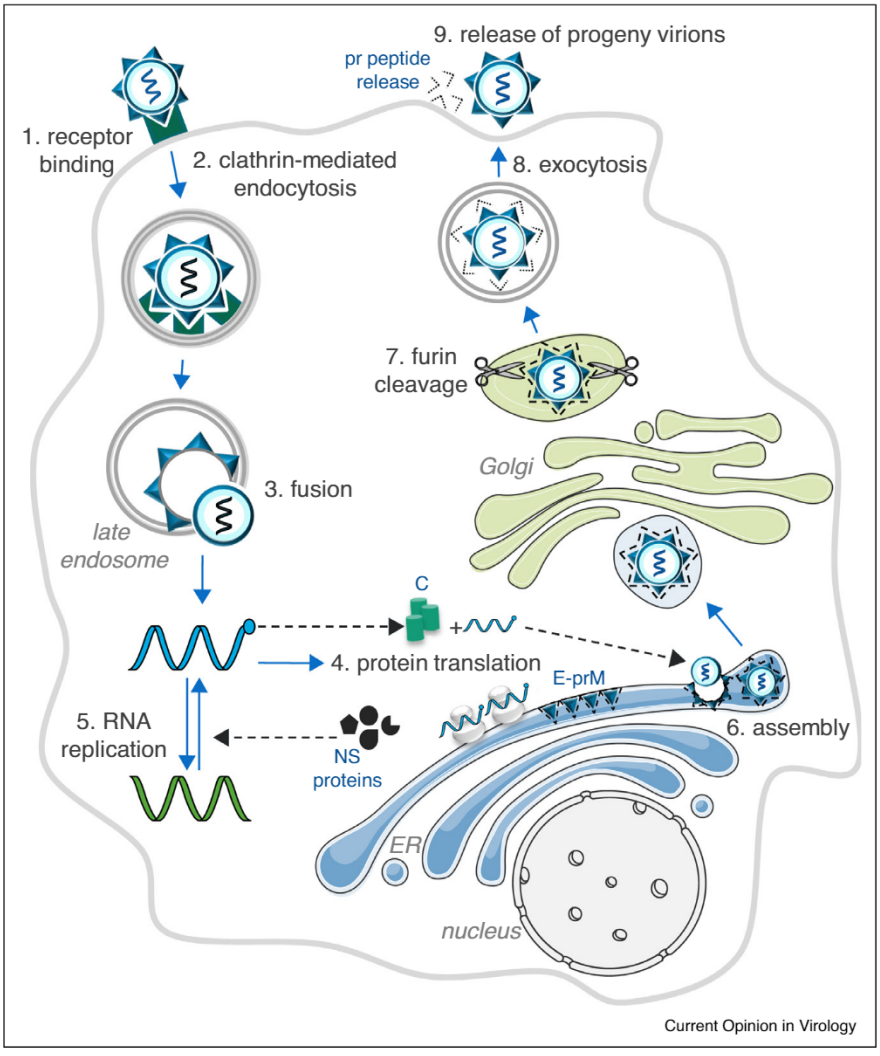
This review will give describe the current status and challenges of antiviral development towards DENV. The chapters are divided on the basis of direct-acting antivirals and host-directed antivirals and the lessons learned and challenges ahead of us.

Antiviral targets

Direct-acting antivirals

Direct-acting antivirals (DAA) are compounds that interact with viral proteins to exert antiviral function [1•]. Generally, DAA offer a promising approach as they specifically target a viral protein and therefore usually show low toxicity and a wide treatment window. A known drawback of DAA is, however, the relatively high risk for resistance development [9]. To date, DENV antiviral research has focused on targeting both the structural and the NS proteins (Table 1). The most extensively studied structural protein as an antiviral target is the E protein,

Figure 1



DENV replication cycle.
DENV infection is initiated by binding of the virus to host-cell receptors (1). The virus is then internalised via clathrin-mediated endocytosis (2) and the low pH in the endosome triggers the membrane fusion reaction (3). Upon membrane fusion and nucleocapsid uncoating, the viral genome is translated into one polyprotein, which is cleaved into structural and NS proteins (4). The structural proteins envelope (E) and premembrane (prM) are translocated to the ER. The NS proteins enable RNA replication, including the production of positive (blue) and negative (green) sense single-stranded RNA copies (5). Genomic RNA (blue) is packed by capsid proteins and the nucleocapsid buds into the ER lumen to form an enveloped immature virion (6). Immature virions are transported through the secretory pathway, where cleavage of prM to M occurs (7). Finally, mature virus particles are released via exocytosis (8,9). Figure was adjusted from Ref. [8].

which plays an essential role in virus cell entry (Figure 1, Table 1) [10•]. The two most studied NS proteins are NS5 and NS3. The NS5 protein is the largest and most conserved DENV NS protein and functions as the viral RNA-dependent RNA polymerase (RdRP) and has methyltransferase (MTase) activity [11,12]. Whereas both functions have been investigated as antiviral targets, most studies focus on its function as RdRP (Table 1). For NS3, which is also a multifunctional protein, most studies have focused on inhibiting the NS3/NS2B serine protease function (Table 1). Despite the large number of DAA identified using *in vitro* assays very few are validated in mice studies. Furthermore, only one DAA, the RdRp inhibitor balapiravir, has been tested in clinical trials. Unfortunately, however, no differences in plasma viral load, cytokine profile and fever clearance time between the placebo and balapiravir group was observed [13•].

Host-directed antivirals

Viruses hijack/interfere with numerous cellular pathways to create a favourable environment for virus replication. Thus, the identification of compounds interfering with

Table 1

Direct-acting antivirals reported until May 2020

Drug	Target	Mechanism	Reference
1662G07 and analogs	E protein	Binding to dimeric prefusion form of E protein and prevention of fusion	[15]
DET2/DET4	E protein	Inhibition of virus binding and entry	[16,17]
Curdlan sulfate	E protein	Inhibition of virus binding and entry	[18]
1OAN1	E protein	Fusion inhibitor	[19]
DN57opt	E protein	Fusion inhibitor	[19]
DN59	E protein	Fusion inhibitor	[20]
Compound-6[a]	E protein	Fusion inhibitor	[21]
Compound 5a	E protein	Fusion inhibitor	[22]
Rolitetracycline	E protein	Fusion inhibitor	[23]
Doxycycline	E protein	Fusion inhibitor	[23]
NITD448	E protein	Fusion inhibitor	[24]
A5	E protein	Fusion inhibitor	[22]
1662G07 and derivatives	E protein	Fusion inhibitor	[15]
E 419-447 peptides	E protein	Fusion inhibitor	[25]
P02	E protein	Inhibitor of virus entry	[26]
HHA, GNA, UDA	E protein	Inhibitor of virus binding and entry	[27]
Pradimicin-S	E protein	Inhibitor of virus binding and entry	[27]
Fucoidan	E protein	Inhibitor of virus binding and entry	[28]
Sulfated K5 polysaccharide	E protein	Inhibitor of virus entry	[29]
Chondroitin sulfate E	E protein	Inhibitor of virus entry	[30]
PI-88[a]	E protein	Inhibitor of virus binding and entry	[31]
MLH40	E protein	Inhibitor of virus entry	[32]
BP34610	E protein	Inhibitor of virus entry	[33••]
ST-148[a]	C protein	Capsid protein inhibitor	[34,35]
VGTI-A3/VGTI-A3-03	C protein	Capsid protein inhibitor	[36]
Pep14-23	C protein	Inhibition of Interaction of C protein with lipid droplets	[37]
7-deaza-2′-C-methyl-adenosine	NS5 RdRP	Inhibitor of viral replication	[38,39]
INX-08189	NS5 RdRP	Inhibitor of viral replication	[40]
R1479	NS5 RdRP	Inhibitor of viral replication	[41]
7DMA[a]	NS5 RdRP	Inhibitor of viral replication	[39]
Compound 18c	NS5 RdRP	Inhibitor of viral replication	[42]
Compound 13	NS5 RdRP	Inhibitor of viral replication	[43]
BCX4430	NS5 RdRP	Inhibitor of viral replication	[44]
Balapiravir[a,b]	NS5 RdRP	Inhibitor of viral replication	[41,45]
NITD008[a]	NS5 RdRP	Adenosine nucleoside	[46]
2′-C-methylcytidine	NS5 RdRP	Inhibitor of replication	[47]
NITD203[a]	NS5 RdRP	Inhibitor of replication	[48]
Azidothymidine-based triazoles	NS5 MTase	Inhibitor of viral RNA capping	[49]
Compound 10	NS5 MTase	Inhibitor of viral RNA capping	[50]
BG-323[a]	NS5 MTase	Inhibitor of viral RNA capping	[51,52]
NSC 12155	NS5 MTase	Inhibitor of viral RNA capping	[53]
Suramin[a]	NS3 helicase	NS3 helicase inhibition	[54]
ST-610[a]	NS3 helicase	NS3 helicase inhibition	[55]
Compound 25	NS3 helicase	NS3 helicase inhibition	[56]
Compound 7	NS3 helicase	NS3 helicase inhibition	[57]
Nelfinavir	NS2B/NS3 protease	Protease inhibition	[58]
Carnosine	NS2B/NS3 protease	Protease inhibition	[59]
Palmatine	NS2B/NS3 protease	Protease inhibition	[60]
Thiazolidinone-peptide hybrids	NS2B/NS3 protease	Protease inhibition	[61]
Compound 32	NS2B/NS3 protease	Protease inhibition	[62]
Compound 1	NS2B/NS3 protease	Protease inhibition	[63]
166347	NS2B/NS3 protease	Protease inhibition	[64]
ARDP0006 and ARDP0009	NS2B/NS3 protease	Protease inhibition	[65]
Compound 7n	NS2B/NS3 protease	Protease inhibition	[66]
Diaryl(thio)ethers	NS2B/NS3 protease	Protease inhibition	[67]
Compound C,D and F	NS2B/NS3 protease	Protease inhibition	[68]
Compound 1-6 and 8	NS2B/NS3 protease	Protease inhibition	[69]
Ltc1	NS2B/NS3 protease	Protease inhibition	[70]
BP13944	NS2B/NS3 protease	Protease inhibition	[71]
BP2109	NS2B/NS3 protease	Protease inhibition	[72]
Retrocyclin 1	NS2B/NS3 protease	Protease inhibition	[73]
MB21	NS2B/NS3 protease	Protease inhibition	[74]

Table 1 (*Continued*)

Drug	Target	Mechanism	Reference
Policresulen	NS2B/NS3 protease	Protease inhibition and destabilization	[75]
Compound 45a	NS2B/NS3 protease	Protease inhibition	[76]
Compound 104	NS2B/NS3 protease	Protease inhibition	[77]
Compound 14	NS2B/NS3 protease	Protease inhibition	[78]
SK-12	NS2B/NS3 protease	Inhibition of interaction between NS2B and NS3	[79]
Ivermectin	NS5	Inhibition of NS5 interaction with importin α and β	[80–83]
	NS3 helicase	NS3 helicase inhibition	
	NS2B/NS3 protease	Protease inhibition	
Compound-B	NS4A	Inhibitor of viral replication	[84]
NITD-618[a]	NS4B	NS4B inhibition	[85]
Lycorine	2k peptide	unknown	[86]
AM404	NS4B	NS4B inhibition	[87]
Compound 14a[a]	NS4B	NS4B inhibition	[88,89]
SDM25N	NS4B	Inhibition of IFN signaling	[90]
Dasatinib	NS4B	NS4B inhibition	[91]
AZD0530	NS4B	NS4B inhibition	[91]

[a] Tested *in vivo*.
[b] Tested in clinical trials.

these cellular pathways, referred to has host-directed antivirals (HDA), is a promising antiviral strategy [14•]. Furthermore, as different (arbo)viruses often hijack/exploit similar host factors, HDA have a great potential for broad-spectrum treatment [6•]. Furthermore, HDA bear a lower risk of resistance development, which may increase their efficacy. However, HDA typically have a smaller toxicity and efficacy window compared to DAA as their function may also interfere with cell homeostasis [9,14•]. Various HDA antivirals have been identified, which target different stages of the viral replication cycle (Table 2). The most studied cellular target is α-glucosidase, which facilitates proper protein folding and maturation [1•]. Moreover, several studies describe the inhibition of the cellular inosine monophosphate dehydrogenase, which has an essential function in nucleotide biosynthesis and thus viral replication (Table 2) [13•]. Few HDA have been tested in clinical trials. An example is the α-glucosidase inhibitor UV-4B, which showed a good safety profile up to a concentration of 1000 mg (NCT02061358) [1•]. However, a follow-up study investigating the pharmacokinetics of UV-4B in healthy volunteers has been terminated (NCT02696291).

Lessons learned and challenges to be faced

Identification of antiviral compounds

In the last decades, various screening methods have been used to identify new potential DAA and HDA to treat DENV infection. Those include biophysical, biochemical and cell-based approaches [10•]. Moreover, open access data bases (e.g. DenHunt and DenvInt) have been published that map dengue–human and dengue–mosquito protein interactions [185,186••]. These data bases provide a common ground for the identification of new inhibitors. Furthermore, the innovation in the X-ray structures of various DENV proteins allows for computational screening techniques such as *in silico* compound docking and structure based drug design [1•]. Many of the identified compounds uncovered by these approaches are currently only evaluated *in vitro*. Further testing is halted due to various reasons including the efficacy, specificity, toxicity, and stability of the compound. What are important criteria for further development? First, antiviral activity should be seen in a panel of human cell lines and preferably in human primary cells that are important during natural DENV infection. Those may include but are not restricted to human hepatic cell lines such as Huh7 cells or U2OS cells as well as primary human peripheral blood mononuclear cells and human monocyte-derived macrophages, which are all cellular targets during natural dengue virus infection [41,154,187]. Additionally, newly developed organoid cultures, such as 3 dimensional liver organoids, may be helpful in the future to evaluate antiviral activity in a more complex *in vitro* model [188••]. Second, antiviral activity is ideally detected for all serotypes and preferably confirmed for multiple DENV strains within a serotype [10•]. Nevertheless, DENV inhibitors which are protective towards two or three serotypes should not be neglected for further testing. Serotype-specific treatment may help to treat serotype-confirmed DENV patients and when more antiviral drugs are available it may be possible to achieve a pan-protective effect via drug combinational therapy. Third, limited cellular cytotoxicity should be seen. In cellular cytotoxicity three parameters are important: metabolic activity, proliferation capacity and cell death. Multiple assays should be used to assess cellular cytotoxicity for example, MTT/ATPlight to assess metabolic activity and for example trypan blue staining to assess proliferation/cell death [189•]. Moreover, novel techniques such as

Table 2

Host-directed antivirals reported until May 2020

Drug	Target	Mechanism	Refs.
Construct 13.4	DC-SIGN receptor	Inhibition of entry	[92]
Duramycin	TIM1 receptor	Inhibition of entry	[93]
Met-RANTES	CC-chemokine receptor CCR5	Inhibition of replication	[94]
UK-484900	CC-chemokine receptor CCR5	Inhibition of replication	[94]
Prochlorperazine[a]	Dopamine receptor D2 antagonist Clathrin lattices formation	Clathrin-mediated inhibition	[95]
Bromocriptine[a]	Dopamine receptors D2 and D3 agonist	Inhibition of replication	[96]
SKI-417616	Dopamine receptor D4 antagonist	Inhibition of replication	[97]
Chloroquine[a,b]	Low-pH dependent entry steps and furin-dependent virus maturation Anti-inflammatory properties	Inhibition of fusion and maturation	[98–102]
Compound 45 Compound 46	Furin	Mature DENV particle production	[103]
Luteolin	Furin	Mature DENV particle production	[104]
C75	Fatty-acid synthase	Inhibition of replication	[105,106]
Cerulenin	Fatty-acid synthase	Inhibition of replication	[105]
Orlistat	Fatty-acid synthase	Inhibition of replication	[107]
Methyl-β-cyclodextrin	Cholesterol biosynthesis	Inhibition of replication	[108]
Nordihydroguaiaretic acid	Lipid droplet formation in cells/fatty acid biosynthesis	Inhibition of assembly and replication	[109]
U18666A	Cholesterol transport and biosynthesis	Inhibition of replication	[110]
Lovastatin[a,b]	HMG-CoA reductase	Inhibition of entry and assembly	[111–114]
Fluvastatin, atorvastatin, pravastatin, simvastatin	HMG-CoA reductase	Inhibition of replication	[115]
Hymeglusin	HMG-CoA reductase	Inhibition of replication	[116]
Zaragozic acid	Squalene synthetase	Inhibition of replication	[116]
4-HPR[a]	Interaction between viral proteins and IMPa/b1 eIF2α phosphorylation	Inhibition of replication	[117–119]
AR-12[a]	3-phosphoinositide-dependent kinase 1	Inhibition of replication	[120]
MG-132	Proteasome pathway	Inhibition of replication	[121,122]
Lactacystin	Proteasome pathway	Inhibition of replication	[122]
ALLN	Proteasome pathway	Inhibition of replication	[121]
Bortezomib[a]	Proteasome pathway	Inhibition of egress	[123]
IU1	Proteasome-associated deubiquitinating enzyme USP14	Inhibition of replication	[124]
UBEI-41	Ubiquitin-proteasome pathway	Inhibition of replication	[125]
β-lactone	Ubiquitin-proteasome pathway	Inhibition of egress	[123]
PF-429242	Site 1 protease	Inhibition of replication	[126]
Ribavirin[a]	Inosine monophosphate dehydrogenase	Guanosine depletion	[127–130]
Mycophenolic acid	Inosine monophosphate dehydrogenase	Guanosine depletion	[130,131]
ETAR	Inosine monophosphate dehydrogenase	Inhibition of replication	[132]
IM18	Inosine monophosphate dehydrogenase	Inhibition of replication	[132]
N-allyl-acridone	Inosine monophosphate dehydrogenase	Inhibition of replication	[133]
NITD-982	Dihydroorotate dehydrogenase	Inhibition of pyrimidine biosynthesis	[134]
Brequinar	Dihydroorotate dehydrogenase	Guanosine depletion RNA synthesis Viral assembly/release	[135]
Compound 3A	Dihydroorotate dehydrogenase	Depletion of pyrimidine pools	[136]
Amodiaquine	Heme-polymerase activity	Generation of free heme/inhibition of replication	[137]
Cyclosporine	Interaction of cyclophilin A and NS5	Protein folding/replication	[138,139]
Sunitinib and erlotinib[a]	AAK1 and GAK kinases	Inhibition of replication	[140••]
12r	GAK kinase	Inhibition of replication	[140••,141]
AZD0530	Fyn kinase	Inhibition of SRC FYN kinases	[91,142]
Dasatinib	Fyn kinase	Inhibition of SRC FYN kinases Inhibition of egress	[91,142]
SFV785	NTRK1 and MAPKAPK5 kinase	Inhibition of replication	[143]
SB203580[a]	P38 MAPK	Inhibition of replication	[144]
2-deoxy-D-glucose (2DG)	Hexokinase	Inhibition of replication	[145]
GNF-2	Abl kinase/viral E protein	Inhibition of entry and replication	[146]
Imatinib	BCR-Abl kinase	Inhibition of replication	[146]
Castanospermine[a]	α-glucosidase	prM and E folding interference Misfolding of NS1	[147,148]
Celgosivir[a,b]	α-glucosidase	Accumulation of E and NS1 in ER	[38,149–151]
Deoxynojirimycin	α-glucosidase	Inhibition of budding from ER	[152]

Table 2 (*Continued*)

Drug	Target	Mechanism	Refs.
NN-DNJ[a]	α-glucosidase	prM and E folding interference Reduction in secretion of E and NS1 protein	[38,153]
NB-DNJ[a]	α-glucosidase	Reduction in secretion of E and NS1 protein	[154]
OSL-9511	α-glucosidase	Reduction in secretion of E and NS1 protein	[155]
SP169 and SP173 PBDNJ081	α-glucosidase	Interference with viral glycoprotein folding	[155]
PBDNJ083 PBDNJ084	α-glucosidase	Interference with viral glycoprotein folding	[156]
Compound 31	α-glucosidase	Interference with viral glycoprotein folding	[157]
UV-4[a]	α-glucosidase	Interference with viral glycoprotein folding	[158]
UV-4B[a,b]	α-glucosidase	Interference with viral glycoprotein folding	[159]
UV-12[a]	α-glucosidase	Interference with viral glycoprotein folding	[160]
CM-9-78/CM-10-18[a]	α-glucosidase	Interference with viral glycoprotein folding	[161,162]
Kotalanol	α-glucosidase	Interference with viral glycoprotein folding	[163]
Compound 36	α-glucosidase	Interference with viral glycoprotein folding	[164]
Zaragozic acid	Squalene enzyme	Inhibition of replication	[116]
Prednisolone[b]	Anti-inflammatory and anti-hemorrhagic activity		[165,166]
Dexamethasone	Anti-inflammatory and immunosuppressive activity	Prevention of thrombocytopaenia	[167]
Schisandrin A[a]	STAT1/2 mediated antiviral interferon responses	Inhibition of replication	[168]
Celastrol	IFN-expression	Inhibition of replication	[169]
Salidroside	Rig-I	Inhibition of viral proteins synthesis	[170]
Hydroxychloroquine	Induction of IFN-β, AP-1 and NFkB pathways and production of reactive oxygen species	Inhibition of replication	[171]
Asunaprevir	Mitochondrial antiviral-signaling protein pathways	Inhibition of replication	[172]
U. guianesis extracts UGL and UGB	Chemokine/cytokine production	Inhibition of replication	[173]
U. tomentosa	chemokine/cytokine production	Inhibition of replication	[174]
Minocycline	ERK 1/2 and IFN-α	Inhibition of replication	[175]
U0126	ERK 1/2	Inhibition of replication	[176]
Cavinafungin	ER-associated signal peptidase	Inhibition of replication	[177]
Compound 16d	DEAD-box polypeptide 3 (DDX3)	Inhibition of replication	[178]
Lanatoside C	NA^+-K^+-ATPase pump	Inhibition of RNA synthesis	[179]
Leptomycin B	Exportin CRM1	Inhibition of replication	[180]
Compound 2 Compound 3	S-adenosylhomocysteine hydrolase	Inhibition of replication	[181]
Oxamate	Lactate dehydrogenase	Inhibition of replication	[145]
Geneticin	80S ribosome	Inhibition of protein translation	[182]
Lactimidomycin	Translation elongation	Inhibition of replication	[183]
Ketotifen[a]	Mast cell modulator	Reduction of vascular leakage	[184]
Cromolyn[a]	Mast cell modulator	Reduction of vascular leakage	[184]
Montelukast[a]	Mast cell modulator	Reduction of vascular leakage	[184]

[a] Tested *in vivo*.
[b] Tested in clinical trials.

cellular thermal shift assays and 3 dimensional organoids of the liver may help to gain a more complex and specific picture of the cytotoxicity profile of newly discovered drugs in the future [188••,190,191]. Fourth, viral resistance should not develop quickly. Lastly, the stability of the compound is important. It is crucial for an antiviral compound to be stable in order to be efficiently absorbed by and distributed throughout the body [10•]. Limited stability can lead to an early degradation in the gastrointestinal tract, liver or kidney and thereby significantly reduces the systemic drug concentration. An example of an *in vitro* assay to test drug stability is the hepatic microsome assay. Here, subcellular liver fractions containing drug-metabolizing enzymes are used to investigate metabolic degradation of a drug [192].

Altogether, it appears challenging to identify compounds with the proper characteristics. More in-depth research in the virus–host interactions that occur during viral infection and/or chemical modification of already identified compounds may aid in development of compounds that fulfil the required criteria.

Important considerations regarding *in vivo* studies

When a compound exhibits an appropriate *in vitro* profile, the *in vivo* efficacy is tested in mice. The AG129 model is

still considered the best model to study DENV infection, due to the lack of more representative small animal DENV disease models. AG129 mice are deficient in the interferon I and II receptors and therefore induce high DENV viremia levels and high levels of pro-inflammatory cytokines, which leads to the development of thrombocytopenia, vascular leakage and death [193]. There are two distinct AG129 mouse models, the lethal and the non-lethal mouse model. This is based on the virus strain used and the dose applied [193]. Whereas the lethal mouse model has the advantage of high systemic viremia and severe disease symptoms relevant to human DENV infection, the nonlethal model has lower viremia yet therefore allows for the investigation of time of viremia clearance [150,194•,195••]. The lethal model is most often used to test antiviral efficacy. Here, antiviral efficacy is most often determined on the basis of a reduction in peak viremia [34,38,48,196]. In humans, antiviral therapy is, however, most likely prescribed when peak viremia is established or is starting to decline [194•]. Furthermore, the majority of drugs with a potent antiviral *in vitro* profile that have been tested in clinical trials show low or no efficacy when peak viremia is already established [99,113,151] This was also reported for celgosivir, after which the researchers decided to perform a follow-up study in a non-lethal AG129 model. The efficacy of celgosivir was found improved after adjusting the treatment regime from two to four times a day even when treatment started during peak viremia [150]. On the basis of these findings celgosivir is currently evaluated in a newly approved clinical trial using an adjusted treatment regime (NCT02569827). In summary, both AG129 models are used to study antiviral efficacy but we favour the non-lethal AG129 model as it allows you to study the time needed to resolve viremia, which is more representative for the clinical situation.

Next to measuring viremia, a new non-invasive imaging technique is currently being evaluated to detect infection based on inflammation *in vivo*. ^{18}F-fluorodeoxyflucose (FDG)-PET is an imaging probe detecting abnormal glucose metabolism and was already successfully used in DENV-infected AG219 mice to asses antiviral properties of celgosivir. The authors showed a significant reduction in ^{18}F-FDP uptake, corresponding to reduced inflammation, from two days post-infection in various organs such as spleen, liver and stomach [197,198]. Thus, this method could serve as an additional, non-invasive tool to evaluate drug efficacy in preclinical trials by evaluating reduced inflammation.

Lessons learned from approved antivirals and challenges ahead of us

From the 200 human viruses that have been identified to date, only 9 can be treated by licenced antiviral compounds [199]. These viruses comprise human immunodeficiency virus, hepatitis B virus, hepatitis C virus (HCV), herpes virus, influenza virus, human cytomegalovirus, varicella-zoster virus, respiratory syncytial virus and human papillomavirus. Many approved treatment regimens are based on combinational therapies, using antiviral drugs that target different steps of the virus replication cycle [200]. This is merely done to avoid resistance development and to enhance antiviral efficacy.

For DENV, some studies have applied combinational drug testing by combining ribavirin with either the α-glucosidase inhibitor CM-10-18, the nucleoside analog INX-08189 or the E protein inhibitor BP34610 [33••,161,200]. The studies demonstrated that combination treatment lead to an enhancement of the antiviral efficacy *in vitro* (ribavirin with CM-10-18, INX-08189 or BP34610) and *in vivo* (ribavirin with CM-10-18). Moreover, combination of CM-10-18 with ribavirin led to a synergistic antiviral effect, even when CM-10-18 was added in a subeffective dose [161]. Given the lessons learned from other viruses and the initial positive results for DENV, more emphasis should be on evaluating the antiviral efficacy of distinct cocktails of drugs.

Concluding remarks

It is challenging to develop a safe and effective antiviral compound towards DENV. Antiviral drug development is hampered by the need to identify a compound with pan-protective antiviral properties, low toxicity, low chance of viral resistance, and proper stability to ensure absorption and distribution. Continuous innovations in screening approaches, X-ray structures and openly accessible databases represent a promising base for the identification of new drugs, yet further evaluation of existing drugs is also warranted. Combination treatment seems to be the most promising antiviral approach to overcome the current challenges of anti-DENV drug development. Combining two or more DAA seems most plausible, especially given the success in HCV treatment [201]. However, combining DAA with HDA might be best as this reduces the risk of viral resistance and increases the chance of a pan-protective effect.

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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